CLAIMS

What is claimed is:

[00114] 1. A process for isolating plasmid DNA comprising the steps of:

[00115] (a) lysing cells containing said plasmid DNA with a lysis agent, thereby forming a lysate;

[00116] (b) treating said lysate with a high salt agent, thereby forming a treated solution; and

[00117] (c) purifying said treated solution to provide isolated plasmid DNA.

[00118] 2. The process of claim 1, wherein said plasmid DNA is a mixture of supercoiled plasmid DNA, nicked circle plasmid DNA, and linearized plasmid DNA.

[00119] 3. The process of claim 1, wherein said process does not involve the use of RNase.

[00120] 4. The process of claim 1, wherein said high salt agent is capable of precipitating a significant portion of any RNA molecules from said cells.

[00121] 5. The process of claim 1, wherein said high salt agent comprises one or more salts at a pH above approximately 5.5.

[00122] 6. The process of claim 5, wherein said high salt agent comprises a mixture of 1M potassium acetate and 7M ammonium acetate at a pH between 7.0 and 9.0.

[00123] 7. The process of claim 6, wherein said lysate is treated with said high salt agent for at least six hours at approximately four degrees Celsius.

[00124] 8. The process of claim 1, wherein said isolated plasmid DNA is pharmaceutical-grade plasmid DNA suitable for administration to humans.

[00125] 9. The process of claim 1, wherein at least 100 milligrams of said isolated plasmid DNA is obtained.

[00126] 10. A process for isolating plasmid DNA comprising the steps of:

[00127] (a) resuspending cells in approximately 50 mM of Tris at a pH of about 8.0 and approximately 10 mM EDTA(Na)2;

[00128] (b) lysing cells containing said plasmid DNA with a lysis agent comprising an approximately equal volume of 0.2N sodium hydroxide in 1% SDS, thereby forming a lysate;

[00129] (c) treating said lysate with a high salt agent comprises a mixture of 1M potassium acetate and 7M ammonium acetate at a pH between 7.0 and 9.0, thereby forming a high salt solution; and

[00130] (c) purifying said treated solution to provide isolated plasmid DNA.

[00131] 11. The process of claim 10, wherein said isolated plasmid DNA is pharmaceutical-grade plasmid DNA suitable for administration to humans.

[00132] 12. The process of claim 10, wherein at least 100 milligrams of said isolated plasmid DNA is obtained.

[00133] 13. In the process of claim 10 for isolating plasmid DNA from lysate of a cell containing said plasmid DNA, wherein the improvement comprises treating said lysate with a high salt agent capable of precipitating non-plasmid DNA cellular components contained in said lysate.

[00134] 14. The process of claim 13, wherein said high salt agent is capable of precipitating a significant portion of any RNA molecules from said cells.

[00135] 15. The process of claim 13, wherein said high salt agent comprises one or more salts at a pH above approximately 5.5.

[00136] 16. The process of claim 15, wherein said high salt agent comprises a mixture of 1M potassium acetate and 7M ammonium acetate at a pH between 7.0 and 9.0.

[00137] 17. The process of claim 16, wherein said lysate is treated with said high salt agent for at least six hours at approximately four degrees Celsius.

[00138] 18. A process for isolating plasmid DNA comprising the steps of:

[00139] (a) lysing cells containing said plasmid DNA with a lysis agent, thereby forming a lysate; and

[00140] (b) purifying said lysate with anion exchange chromatography using a step gradient, thereby producing isolated plasmid DNA (The process of claim 18, wherein the isolated

plasmid DNA is enriched with at least 80% supercoiled plasmid DNA).

[00141] 19. The process of claim 18, wherein said isolated plasmid DNA is pharmaceutical-grade plasmid DNA suitable for administration to humans.

[00142] 20. The process of claim 18, wherein at least 100 milligrams of said isolated plasmid DNA is obtained.

[00143] 21. The process of claim 18, wherein said anion exchange chromatography is performed with a resin having a particle size of 20-40 microns.

[00144] 22. The process of claim 22, wherein said anion exchange chromatography has a plasmid DNA binding capacity of about 1.5 mg of plasmid per mL of resin.

[00145] 23. The process of claim 18, wherein said anion exchange chromatography is performed with a Fractogel EMD TMAE(650-S) resin.

[00146] 24. The process of claim 18 for isolating plasmid DNA from lysate of a cell containing said plasmid DNA, wherein the improvement comprises purifying said lysate with anion exchange chromatography using a step gradient, thereby producing isolated plasmid DNA enriched with at least 80% supercoiled plasmid DNA.

[00147] 25. The process of claim 24, wherein said anion exchange chromatography is performed with a resin having a particle size of 20-40 microns.

[00148] 26. The process of claim 24, wherein said anion exchange chromatography has a plasmid DNA binding capacity of about 1.5 mg of plasmid per mL of resin.

[00149] 27. The process of claim 24, wherein said anion exchange chromatography is performed with a Fractogel EMD TMAE(S) resin.

[00150] 28. A process for isolating plasmid DNA comprising the steps of:

[00151] (a) lysing cells containing said plasmid DNA with a lysis agent, thereby forming a lysate; and

[00152] (b) using hydrophobic interaction chromatography to purify said lysate, thereby providing isolated plasmid DNA.

[00153] 29. The process of claim 27, wherein said hydrophobic interaction chromatography is performed with at least 1.6M ammonium sulfate.

[00154] 30. The process of claim 28, wherein said hydrophobic interaction chromatography is performed with an Octyl Sepharose 4 FF resin.

[00155] 31. The process of claim 28, wherein said isolated plasmid DNA is pharmaceutical-grade plasmid DNA suitable for administration to humans.

[00156] 32. The process of claim 28, wherein at least 100 milligrams of said isolated plasmid DNA is obtained.

[00157] 33. The process of claim 28, wherein said plasmid DNA is not precipitated and wherein said process involves no linear gradients and uses no organic solvents.

[00158] 34. The process of claim 28, wherein said isolated plasmid DNA is substantially free of endotoxins and host cell chromosomal DNA.

[00159] 35. The process of claim 28, wherein said plasmid DNA is not exposed to acidic pH or elevated temperatures.

[00160] 36. The process of claim 28, wherein said isolated plasmid DNA is produced in a yield of at least 60%.

[00161] 37. The process of claim 28, wherein said hydrophobic interaction chromatography is performed in an aqueous solution containing a high concentration of salt.

[00162] 38. The process of claim 37, wherein said salt is ammonium sulfate.

[00163] 39. The process of claim 28, wherein said cells are recombinant *E. coli* cells.

[00164] 40. In a process for isolating plasmid DNA from a lysate of a cell containing said plasmid DNA, wherein the improvement comprises using hydrophobic interaction

chromatography to purify said lysate, thereby providing isolated plasmid DNA.

[00165] 41. The process of claim 42, wherein said hydrophobic interaction chromatography is performed with at least 1.6M ammonium sulfate.

[00166] 42. The process of claim 42, wherein said hydrophobic interaction chromatography is performed with an Octyl Sepharose 4 FF resin.

[00167] 43. The process of claim 42, wherein said hydrophobic interaction chromatography is performed in an aqueous solution containing a high concentration of salt.

[00168] 44. The process of claim 42, wherein said salt is ammonium sulfate.

[00169] 45. A device for isolating plasmid DNA from cells containing said plasmid DNA, comprising:

[00170] (a) a means for providing fast cell resuspension in a semi-continuous mode;

[00171] (b) a means for providing mixing and cell lysis in a continuous flow mode; and

[00172] (c) a means for providing chilling and mixing to denature and precipitate chromosomal DNA, protein, and RNA.

[00173] 46. The device of claim 47, wherein said means for providing mixing and cell lysis in a continuous flow mode comprises an impeller mixer.

[00174] 47. The device of claim 47, wherein said means for providing mixing and cell lysis in a continuous flow mode comprises an in-line static mixer.

[00175] 48. The device of claim 47, wherein said means for providing mixing and cell lysis in a continuous flow mode comprises a lysis coil.

[00176] 49. The device of claim 47, further comprising a means for performing hydrophobic interaction chromatography.

[00177] 50. The device of claim 47, wherein said means for providing chilling and mixing to denature and precipitate

chromosomal DNA, protein, and RNA comprises a chilled jacketed tank.

[00178] 51. A process for isolating plasmid DNA, comprising the steps of:

[00179] (1) fermenting cells containing said plasmid DNA, harvesting said cells, and washing said cells;

[00180] (2) exposing said cells to an alkaline lysis and neutralization agent, thereby forming a lysate;

[00181] (3) performing centrifugation and filtration on said lysate;

[00182] (4) treating said lysate with RNase at about 37 degrees Celsius for about one hour;

[00183] (5) filtrating said lysate and diluting said lysate with 2 volumes of WFI.

[00184] (6) passing said lysate through a Q Sepharose HP resin, a DEAE 650-S resin, and a Phenyl 650-S resin; and

[00185] (7) filtrating the eluate from step 6 to yield the final product of isolated plasmid DNA.

[00186] 52. A process for isolating plasmid DNA, comprising the steps of:

[00187] (a) fermenting cells containing said plasmid DNA, harvesting said cells, and washing said cells;

[00188] (b) exposing said cells to an alkaline lysis and neutralization agent, thereby forming a lysate;

[00189] (c) performing centrifugation or filtration on said lysate and performing a 1.5 volume dilution with WFI on said lysate;

[00190] (d) exposing said lysate to an anionic change resin;

[00191] (e) washing the nicked and/or relaxed circular plasmid, as well as residual RNA, off of the resin with about 0.6 M NaCl;

[00192] (f) eluting the plasmid DNA off of the resin with about 1.9M ammonium sulfite;

[00193] (g) passing the eluate through a hydrophobic interaction chromatography resin; and

[00194] (h) filtrating the eluate to yield a final product of isolated plasmid DNA.